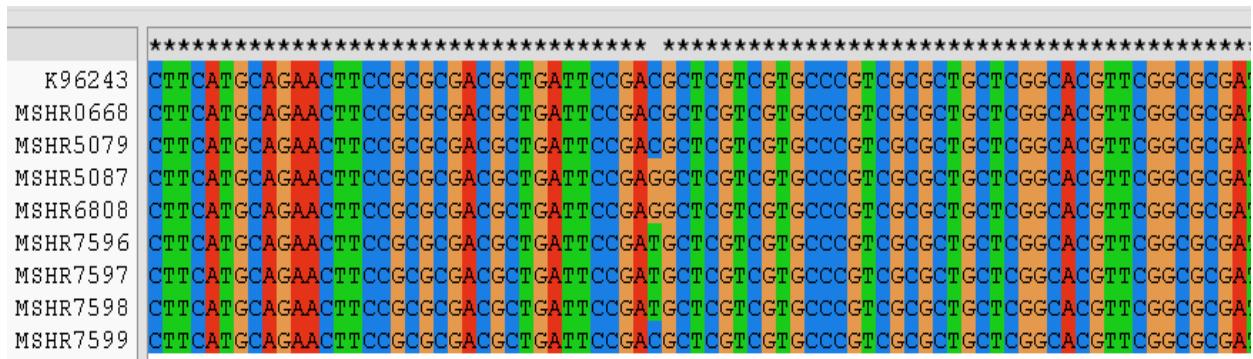


Supplementary Table 1: Minimum inhibitory concentrations for *B. pseudomallei* isolates obtained in this study

Strain	Minimum inhibitory concentration ( $\mu\text{g/ml}$ )							
	AMX	AZM	CAZ	DOX	GEN	KAN	MEM	SXT
MSHR5079	3	16	1.5	1.5	96	24	0.75	0.75
MSHR5084	3	ND	1.5	3	32	16	0.75	0.5
MSHR5087	3	ND	1.5	1.0	1.0	2	0.5	3
MSHR5089	3	ND	1.5	1.5	1.0	2	0.5	4
MSHR5091	6	ND	3	1.5	1.0	1.5	0.75	4
MSHR5093	6	ND	4	1.5	1.0	1.5	1.0	3
MSHR5095	3	ND	1.0	1.5	1.5	2	0.5	0.75
MSHR5097	3	ND	1.0	1.5	1.0	2	0.5	0.5
MSHR5102	3	24	2	3	$\geq 256$	128	2	0.125
MSHR5104	2	ND	0.75	1.0	1.5	2	0.19	0.75
MSHR5105	3	ND	1.5	1.5	1.5	1.5	0.5	4
MSHR5107	3	ND	1.0	1.5	1.0	1.5	0.5	1.0
MSHR6381	3	ND	2	0.5	1.0	2	1.0	3
MSHR6385	3	ND	2	0.75	1.0	1.5	0.75	2
MSHR6386	3	ND	2	0.38	1.0	1.5	0.75	2
MSHR6387	3	ND	3	0.5	1.0	2	0.75	2
MSHR6389	3	ND	2	0.38	1.5	2	0.75	1.0
MSHR6390	3	ND	1.5	0.5	1.0	2	0.75	2
MSHR6392	2	ND	1.5	0.25	0.75	2	0.75	1.5
MSHR6401	3	ND	4	0.75	1.5	2	1.0	1.5
MSHR6404	3	ND	2	0.5	1.5	2	1.0	2
MSHR6408	3	ND	2	0.5	1.0	1.5	0.75	2
MSHR6409	2	ND	1.5	0.5	0.5	2	0.5	2
MSHR6410	3	1.0	2	0.5	0.75	2	0.75	2
MSHR6411	3	ND	2	0.5	1.0	1.5	0.75	2
MSHR6414	1.5	ND	0.75	0.5	0.75	ND	0.5	0.5
MSHR6415	3	ND	1.5	0.75	0.5	2	0.75	3
MSHR6418	3	ND	2	0.75	1.0	1.5	0.75	2
MSHR6419	2	24	2	2	48	24	1.0	2
MSHR6769	2	0.75	1.5	0.75	1.0	2	0.5	0.5
MSHR6774	8	ND	4	0.75	0.5	1.5	1.0	2
MSHR6778	1.5	ND	1.0	0.5	0.5	ND	0.5	2
MSHR6780	3	ND	2	1.5	0.75	2	0.75	1.5
MSHR6785	3	ND	1.5	0.5	0.5	1.5	0.75	3
MSHR6789	3	ND	1.5	0.5	0.5	1.5	0.75	1.5
MSHR6793	3	1.0	1.5	0.75	0.75	2	0.75	2
MSHR6802	3	ND	2	0.5	1.5	2	0.5	1.5
MSHR6803	3	ND	1.5	0.5	0.75	2	0.75	4
MSHR6806	2	ND	3	0.75	1.0	2	1.0	3
MSHR6816	3	24	3	1.0	96	32	0.75	1.5
MSHR6822	3	1.0	1.5	0.5	0.75	2	0.75	1.5
MSHR6824	2	ND	1.0	1.0	16	12	0.75	3

MSHR6828	3	ND	1.0	1.0	0.75	2	1.0	2
MSHR6808	3	1.0	3	0.5	0.75	1.5	1	1-2
MSHR7596	ND	48	ND	ND	$\geq 256$	$\geq 256$	ND	ND
MSHR7597	ND	48	ND	ND	$\geq 256$	$\geq 256$	ND	ND
MSHR7598	ND	32	ND	ND	$\geq 256$	96	ND	ND
MSHR7599	ND	128	3	0.5	$\geq 256$	$\geq 256$	1	1-2

Abbreviations: AMX, amoxicillin-clavulanic acid; AZM, azithromycin; CAZ, ceftazidime; DOX, doxycycline; GEN, gentamicin; KAN, kanamycin; MEM, meropenem; SXT, trimethoprim-sulfamethoxazole; ND, Not determined.



Supplementary Figure 1: Alignment of the *amrB* hyperconserved membrane spanning domain in select *Burkholderia pseudomallei* isolates. An *amrB*-C1102G single nucleotide polymorphism was observed within the Malaysian clinical strains MSHR5087 and MSHR6808, conferring gentamicin sensitivity. In contrast, a novel *amrB*-C1102T single nucleotide polymorphism in the laboratory-generated mutants MSHR7596, 7597 and 7598 resulted in aminoglycoside resistance. The Malaysian clinical strain MSHR5079 shares the wild-type *amrB* sequence (*amrB*-1102C) with other gentamicin-resistant *B. pseudomallei* (K96243 and MSHR0668), and was generated *in vitro* in the MSHR7599 mutant, confirming the role of *amrB*-1102G in gentamicin sensitivity.



Supplementary Figure 2: Panel A shows the location of Malaysian Borneo in relation to other countries within the South East Asian region. Panel B shows the location of the four hospitals mentioned in the study denoted by red dots.